Machine Translation EP0941251B

Title:

USE OF AN IMMUNOGLOBULIN PREPARATION FOR THE PREPARATION OF AN ORAL MEDICAMENT Preventing Hemolytic Uremic Syndrome AND PREPARATION FOR THE SAME

[No abstract translated]

Abstract from WOWO9815578A:

The invention relates to the use of immunoglobulin preparations with high antibody content against EHEC hemolysin and optionally against surface antigens of EHEC hemolysin gene carrying bacteria and against shiga-like toxin II for producing an oral medicament to prevent hemolytic uremic syndrome after EHEC infection and a preparation for the same. The high antibody content can be obtained by a targeted selection of individual donors or by the immunization of donor animals.

Claims:

1. Use of immunoglobulin preparations having an antibody content against EHEC hemolysin according to a titre of = 1:6400 of a solution of 5 g of immunoglobulin per 100 ml of immunoglobulin solution, for the preparation of an orally administrables medicament for the prevention of haemolytic uraemic syndrome following EHEC infection.

2. Use of immunoglobulin preparations according to claim 1, wherein the immunoglobulin preparation additionally has a high antibody content against surface antigens of EHEC hemolysin gene-carrying bacteria.

3. Use of immunoglobulin preparations according to claim 1 or 2, wherein the immunoglobulin preparation additionally has an antibody content against Shiga-like toxin II, according to a titre of = 1:800 of a solution of 5 g of immunoglobulin per 100 ml of immunoglobulin solution .

4. Use of immunoglobulin preparations according to claim 1 to 3, wherein the preparation has the antibody titre by screening of individual donors.

5. Use of immunoglobulin preparations according to claim 1 to 3, wherein the preparation has the antibody titre by immunization of individual donors.

6. Use of immunoglobulin preparations according to claim 1 to 5, wherein the immunoglobulin preparation is obtained from the colostrum or milk of lactating mammals.

7. Use of immunoglobulin preparations according to claim 1 to 6, wherein the immunoglobulin preparation is obtained from the milk or colostrum of cattle.

8. Immunoglobulin preparation obtainable from milk or colostrum of lactating mammals, wherein in that it has an antibody content against EHEC hemolysin according to a titre of = 1:6400 of a solution of 5 g of immunoglobulin per 100 ml of immunoglobulin solution.

9. Immunoglobulin preparation according to claim 8, wherein in that it additionally has a high antibody content against surface antigens of EHEC hemolysin gene-carrying bacteria.

10. Immunoglobulin preparation according to claim 8 or 9, wherein in that it is obtainable from milk or colostrum of cattle.

1. Using Immunglobulinpraeparaten with Antikoerpergehalt against EHEC hemolysin accordance decitex = 1:6400 a solution of infection 5 g of immunoglobulin per 100 ml of immunoglobulin solution for preparing to orally applied drug for preventive treatment of hemolytic uremic syndrome after EHEC.

2. Using Immunglobulinpraparaten accordance to claim 1, wherein the Immunglobulinpraparat also offer high Antikoerpergehalt against Oberflaechenantigene of EHEC hemolysin gene has borne bacteria.

3. Using Immunglobulinpraeparaten accordance to claim 1 or 2, wherein the Immunglobulinpraeparat an additional Antikoerpergehalt against Shiga-like toxin II in accordance with a titer of 1:800 = a solution of 5 g of immunoglobulin per 100 ml of immunoglobulin solution has.

4. Using Immunglobulinpraeparaten accordance to claim 1 to 3, wherein the preparation has the antibody by screening of individual donors.

5. Using Immunglobulinpraeparaten accordance to claim 1 to 3, wherein the compound has the antibody by immunization of the individual donor.

6. Using Immunglobulinpraeparaten accordance to claim 1 to 5, wherein the Immunglobulinpraeparat is extracted from the milk or colostrum milk-mammals.

7. Using Immunglobulinpraeparaten accordance to claim 1 to 6, wherein the Immunglobulinpraeparat is extracted from the milk or colostrum from cattle.

8. Immunglobulinpraeparat, obtained from milk or colostrum milk, mammals, characterized in that it has a Antikoerpergehalt against EHEC hemolysin accordance decitex = 1:6400 a solution of 5 g of immunoglobulin per 100 ml of immunoglobulin solution.

9. Immunglobulinpraeparat according to claim 8, characterized in that it additionally a high Antikoerpergehalt against Oberflaechenantigene of EHEC hemolysin gene has borne bacteria.

10. Immunglobulinpraeparat according to claim 8 or 9, characterized in that it can be obtained from milk or colostrum from cattle.

1. Utilization de preparations d'immunoglobulins ayant une teneur en anticorps contre l'en EHEC hemolysin selon un titre = 1:6400 d'une solution de 5 g d'immunoglobulins pour 100 ml de solution d'immunoglobulins pour la production d'un medicament u Oral usage pour la prevention du syndrome uremique APROS hemolytique et une infection par EHEC.

2. Utilization de preparations d'immunoglobulins selon la revendication 1, caractérisée en ce que la preparation d'immunoglobulins comporte en outre une haute teneur en anticorps contre Of The Antigone de surface de bacteries porteuses d'en gone en EHEC hemolysin.

3. Utilization de preparations d'immunoglobulins selon la revendication 1 ou 2, caractérisée en ce que la preparation d'immunoglobulins presente en outre une teneur en anticorps contre la toxine Shiga II analogue u = 1:800 selon un titre d'une solution de 5 g d'immunoglobulins pour 100 ml de solution d'immunoglobulins.

4. Utilization de preparations d'immunoglobulins selon les revendications 1 u 3, caractérisée en ce que la presente preparation le titre d'anticorps par selection de donneurs individuels.

5. Utilization de preparations d'immunoglobulins selon les revendications 1 u 3, caractérisée en ce que la presente preparation le titre d'anticorps par immunization de donneurs individuels.

6. Utilization de preparations d'immunoglobulins selon les revendications 1 u 5, caractérisée en ce que la preparation d'immunoglobulins u est obtenue partir du lait ou du colostrum de mammifOres produisant du lait.

7. Utilization de preparations d'immunoglobulins selon les revendications 1 u 6, caractérisée en ce que la preparation d'immunoglobulins u est obtenue partir du lait ou du colostrum de vaches.

8. Preparation d'immunoglobulins pouvant ltre u obtenue partir du lait ou du colostrum de mammifOres produisant du lait, caractérisée en ce qu 'elle presente une teneur en anticorps contre l'en EHEC hemolysin selon un titre = 1:6400 d'une solution de 5 g d'immunoglobulins pour 100 ml de solution d'immunoglobulins.

9. Preparation d'immunoglobulins selon la revendication 8, caractérisée en ce qu 'elle presente en outre une haute teneur en anticorps contre Of The Antigone de surface de bacteries porteuses d'en gone en EHEC hemolysin.

10. Preparation d'immunoglobulins selon la revendication 8 ou 9, caractérisée en ce qu 'elle peut ltre u obtenue partir du lait ou du colostrum de vaches.

Machine Translation EP0941251B

Description:

[0001] The present invention relates accordance to claim 1, the use of a Immunglobulinpraeparates high Antikoerpergehalt against EHEC hemolysin for the preparation of an orally applied drug for preventive treatment of hemolytic uremic syndrome after EHEC infection. The subclaims relate to preferred embodiments of the invention and application of a preparation for the above application.

[0002] Escherichia coli (EHEC) are Increasingly recognized as an important pathogen in the human intestinal tract. They have known since 1982 for the cause bloody diarrhea in humans. E. coli O157 is the most common EHEC serotype.

[0003] E. coli 0157 has ever been found, especially in the northern states and particularly in the warmer months of the year. Typical clinical manifestations of these caused by EHEC gastroenteritis are nausea, cramps and bloody diarrhea. The most common cause of outbreaks of infection are contaminated meat and unpasteurized, raw milk. The more cases of EHEC infections have been known, the clearer it became that E. coli 0157 and other EHEC serotypes are a major cause for the occurrence of hemolytic uremic syndrome (HUS) is responsible.

[0004] HUS is a disease that is characterized by the hall marks of an acute hemolytic anemia, thrombocytopenia and nephropathy. HUS is the commonest cause of acute renal failure in childhood. Increasingly it is also diagnosed in adults and elderly. Without adequate treatment, the lethality is high. HUS typically manifests itself during and after a bloody gastroenteritis or colitis, indicated by a sudden, often following interim relief, with further deterioration of general condition and limitation of pale urine. A serious long-term consequence of HUS have kidney complications, which can lead to loss of kidney function. About 10 percent of children with EHEC-associated diarrhea develop hemolytic uremic Snydrom. In about 30 percent of cases this leads to severe kidney failure and HUS in approximately 10 percent of cases, despite the advances in supportive care, such as the timely dialysis and plasma exchange, to death. It is believed today that a large proportion of later renal failure and renal transplantation resulting therefrom caused by an unrecognized HUS in childhood.

[0005] The majority of HUS patients gained EHEC isolates are different than all other E. coli bacteria, large amounts of bacteriophage-encoded verotoxin (Shiga-like toxin, SLT), such as verotoxin 1 (SLT-I) and Verotoxin 2 (SLT-II). The effect of these bacterial toxins endotoxin can not be compared with the classical heat-labile or heat-stable E. coli Enterotoxins or with. Especially the occurrence of verotoxin 2 is often associated with the hemolytic uremic syndrome.

[0006] Typically, when a enteritis used treatments such as antibiotics, can the toxin production and release of verotoxin from EHEC isolates in vitro and may also stimulate in vivo. Motilitaetshemmende antidiarrheals are also contraindicated because they PROMOTE the absorption of toxins from the intestine. All this leads to a Worsening of the prognosis of EHEC disease, and increased risk for developing a HUS. In order to fail the classic, makes for a enteritis applied therapies, and the development of HUS to measure hitherto prevented by effective.

[0007] For some time, it is proposed the use of immunoglobulins for prophylaxis or treatment of HUS and tested.

[0008] In the PCT application WO 89/10139 describes a drug with Antikoerperaktivitaet colostrum from non-immunized mammals, which in Example 13 and antibodies against the Verotoxin 1 (Shiga-like toxin, SLT-I) of EHEC bacteria has.

[0009] Are you one perorally Kolostralmilchpraeparat high Antikoerperaktivitaet against SLT-I, children with EHEC infection with verotoxin-positive detection, as it happens in vivo does not result well in significant reduction or even eradication of these toxins in the. Even 21 days after beginning therapy in a controlled study were detected in 7 of 10 patients still SLT-I in the chair.

[0010] From Pirro, F. (Veterinary Microbiology 43, 131-141, 1995) has been shown that Rinderkolostrum and bovine serum can neutralize the cytotoxic effect of verotoxin in vitro. While all samples contained antibodies against SLT-I, had only 14.7 percent of neutralizing antibodies against SLT-II.

[0011] In animal studies with rabbits was not human immunoglobulin G in a position to prevent the SLT-II associated diseases. The subcutaneous administration of human IgG had only one hours after infection success in preventing the SLT-I, causing diarrhea. After 6 or 24 hours more, no effect could be observed (Havens, L. et al. Microbiol. Immunol. 36, 1077-1085, 1992).

[0012] Even by Finazzi, G. (American Journal of Hematology 41, 165-169, 1992) was shown in patients with Hamolytisch uremic syndrome, showed that the administration of intravenous IgG preparations, no therapeutic success. As a treatment of choice, a complete plasma exchange has been proposed.

[0013] Bitzan, M. et al. (Infection 21, 140-145, 1993) noted in summary that in the majority of HUS patients by the administration of currently available specimens, a Verotoxin-specific IgG is not expected therapeutic effect.

[0014] From Ashenazi S. et al. (Adv.Exp.Med.Biol. 310, 173-177, 1991) has been described that is caused by human milk or colostrum inhibited the adhesion of E. coli in the intestinal wall. The inhibition was caused by free radicals or mannose oligosaccharides of glycoproteins, but not by antibodies of the immunoglobulin fraction, since contains the immunoglobulin fraction of human milk is only very small amounts of Antikorperaktivitat against EHEC. Immunoglobulins from human milk or colostrum are therefore not suitable for the treatment of EHEC infections and for preventive treatment of HUS. On the other hand, the Oligosaccharidfraktion or glycolipids from inadequate human milk or colostrum for the prevention of HUS using inappropriate, as vacancies for such use is woefully. The results are therefore not in the milk or colostrum pay for other mammals, because the composition of the Oligosaccharidfraktion and glycoproteins of human milk is very different from that of the milk of other species.

[0015] From Beutin, L. (Bundesgesundheitsblatt 10/94, 54) were EHEC isolates from stool samples Closer characterized. 67 percent showed one SLT-II-education, 54 percent one SLT-I-formation. All 54 tribes has proved hamolysierend, taking in 89 percent of the EHEC hemolysin

was detected. If E. coli bacteria, which do not belong to the serotype of EHEC, the EHEC hemolysin phenotype formation rarely represented. Increasingly that is found EHEC isolates, although the characteristic EHEC hemolysin, but not SLT-I or SLT-II exhibit.

[0016] By H. Schmidt et al. (Infection and Immunity 63, 1055-1061, 1995) was the plasmid-encoded hemolysin of E. coli described 0157th The EHEC hemolysin was attributed to a clinical significance, as it exists in all tested strains of E. coli 0157 was. The hemolysin are described as important virulence factors of bacteria that can cause among other extraintestinal disease and can react with various cells such as lymphocytes, granulocytes, erythrocytes and kidney cells. Although EHEC hemolysin about 70 percent homology with the E. coli alpha-hemolysin of other tribes has, hemolysin shall not Kreuzneutralisation of the EHEC hemolysin rather than by antibodies against alpha. Patients with hemolytic uremic syndrome are specific antibodies against EHEC hemolysin in their serum. These specific antibodies as well as the widely used antibodies against alpha-hemolysin, but are apparently unable to prevent or cure the HUS.

[0017] The object of this invention is therefore to provide a simple, cost-effective way preparation for prevention of hemolytic uremic syndrome after EHEC infection.

[0018] This object is achieved by the fact that it describes a Immunglobulinpraeparat high Antikoerpergehalt against EHEC hemolysin as in claim 1 used orally.

[0019] Surprisingly it has been found that oral administration of antibodies against this is EHEC hemolysin is able to drastically reduce the stool frequency of patients and prevent the development of HUS. Infection in over 80 percent of the treatments could Erradikation while one of the hemolysin and hemolysin gene-carrying bacteria are detected, then the risk is drastically reduced the development of HUS after EHEC.

[0020] Due to the low won Durchseuchung of people with EHEC bacteria can be any polyvalent Immunglobulinpraeparate with sufficiently high activity against EHEC hemolysin and if necessary against human SLT-II, and is the immunization of a large number of people with bacteria or EHEC hemolysin questionable from an ethical point of view.

[0021] Surprisingly, however, is found in milk and in colostrum-fed cows from selected regions of a much higher content of Antikoerperaktivitaet against EHEC hemolysin and against EHEC hemolysin gene-carrying bacteria, although they are still capable of the toxin or neutralize the toxin gene-carrying bacteria. In the PCT application WO 89/10139 is indeed found that colostrum from non-immunized mammalian antibodies against virtually the entire spectrum of the toxins contained enteropathogenic coli bacteria. In the following years has, however, that are not present in the colostrum of cows immunized against some toxins, not only antibodies, or antibodies in the colostrum fewer cows (eg SLT-II) or even high antibody titers found in vitro, in vivo , were not protective (example SLT-I). All the more surprising was the protective efficacy of immunoglobulins with Antikoerperaktivitaet against EHEC hemolysin and against EHEC hemolysin gene-carrying bacteria.

[0022] preparations, various high antibody titers against EHEC hemolysin can be gained by analyzing the particular individual donations of as-fed cows and selection of such donations with

a high Antikoerpergehalt against EHEC hemolysin. Where appropriate, we can dispense with this screening of individual donors, if you collect the milk of cows from herds that have a high Durchseuchungsgrad with EHEC hemolysin and the natural, therefore, have high antibody activities. Alternatively, you can cows before calving with EHEC-haemolysin and EHEC haemolysin immunized with appropriate gene-carrying bacteria to obtain even at higher antibody activity in the colostrum or milk. Under one here understands the colostrum milk of the first five days after calving is called during the milk from the sixth day after calving than milk. In principle, come for the immunization of donor animals as well as other milk-giving mammals in question. These belong as goats and sheep.

[0023] An inventive Immunglobulinpraeparat can be manufactured eg from bovine colostrum. These colostrum is defatted and subjected to a short-term heating for 20 seconds at 72 degrees C. The Kolostralmagermilch is adjusted to pH 4.4 to 4.7, and separated the precipitated casein by centrifugation or filtration. The Kolostralmolke is concentrated by ultrafiltration and then sterilized. Uses one of the first milk of colostrum, the product contains an immunoglobulin content of 70-80 percent, based on the total protein. The drug can be made up liquid or spray-or freeze-dried. Alternatively, one can be isolated from the whey of cow's milk, as obtained eg in cheese production, by chromatographic techniques by Ultra-/Diafiltration or Faellungsreaktionen the immunoglobulin fraction.

[0024] To increase the Antikoerperaktivitaet against EHEC hemolysin can the cows with EHEC hemolysin, and preferably with additional surfaces antigens EHEC hemolysin gene-carrying bacteria, and particularly preferably additionally immunized with SLT-II. The immunization is preferably performed with inactivated toxins or bacteria and can be given subcutaneously or intranasally in the udder. To improve the success of vaccination is preferably in adjuvant (eg

[0025] Titermax, sales satellite Serva, Heidelberg) of the mixed vaccines.

[0026] The above described or other known means (eg, from eggs of chickens immuniserten) obtained Immunglobulinpraeparat high Antikoerpergehalt against EHEC hemolysin orally in amounts of 2-50 g / day in the form of a powder, dragees, tablets or dissolved in the administered water, milk or other fullness.

[0027] Preferably, the preparation Additionally on a high Antikoerpergehalt against Oberflaechenantigene of EHEC hemolysin gene-carrying bacteria, which can be obtained as described above.

[0028] An advantage, therefore can be a Antigenkoerpergehalt against Shiga-like toxin II (SLT-II).

[0029] In particular, the compound has the following titers, based on an immunoglobulin solution with 5g/100 ml immunoglobulin levels: in the immunoblot by Gunzer et al (J.Clin.Microbiol. 31 (1993), 2604-2610) and Schmidt et al. (Infect.Immun. 63 (1995), 1055-1061) should be the titers against EHEC hemolysin-reactive antibodies = 1:6400. 50 percent of the hemolytic activity of the EHEC hemolysin to erythrocyte according to Bauer et al. (Infect.Immun. 64 (1996), 167-175) should be neutralized with a dilution of the immunoglobulin

solution = 1:50. The titer of neutralizing antibodies ggen SLT-II, measured in standard neutralization accordance Schmidt et al. (Infect.Immun. 61 (1993), 534-543), should be = 1:800.

[0030] The preparation is particularly indicated for bloody-frequency, cramping abdominal pain associated with acute diarrhea, especially children and older people with known or suspected EHEC infection, to prevent the subsequent development of HUS.

[0031] The invention is further illustrated by the following example.

Sample

[0032] Of 36 children with evidence of EHEC infection were children 18, 14 days long, each with g 3 times 7 of a Immunglobulinpraeparates from colostrum with high Antikoerpergehalt against EHEC hemolysin and SLT-II treated. The control group received the same amount of beef gelatin as placebo. The patients were monitored for a further 7 days.

[0033] The Immunglobulinpraeparat was extracted from colostrum from cows from a closed herd with high prevalence of EHEC. These include the first milk of the colostrum was defatted and subjected to a short-term heating for 20 seconds at 72 degrees C. The Kolostralmagermilch was adjusted to pH 4.5 and separated the precipitated casein by filtration. The Kolostralmolke was concentrated by ultrafiltration, sterile filtration and freeze-dried. The product contained in immunoglobulin share of 75 percent, based on the total protein. In the immunoblot allowed himself to prove to a dilution of 1:12800 against EHEC hemolysin-reacting antibodies. 50 of the hemolytic activity of the EHEC hemolysin to erythrocyte percent could still be neutralized by a dilution of 1:100. The levels of neutralizing antibodies against SLT-II was still detectable in a dilution of 1:1600.

[0034] After completion of the study, the average stool frequency in the treatment group at 1 chair per day. In the placebo group 3 chairs per day were observed.

[0035] In the treatment group were EHEC hemolysin gene-carrying bacteria, only around 11 percent in the placebo group, however, in about 43 percent of the patients studied found.

[0036] In the treatment group were detected after 21 days no SLT-II gene-carrying bacteria more in the placebo group has been observed however that even in 2 of 5 patients.

[0037] In the treatment with the inventive Immunglobulinpraeparat was observed no HUS. Both the elimination of the EHEC hemolysin gene-carrying and the SLT-II gene-carrying bacteria and the consequent significant reduction in stool frequency dramatically reduce the risk to the development of HUS.